

Research Highlights

DOI: 10.1039/b706143a

Micromechanical control of cell–cell interactions

Cells in a tissue can communicate either through direct contact or exchange of soluble factors. Such cell–cell interactions are a requirement for the development and function of living tissues, and the understanding of the spatial and temporal aspect of cell communication is of fundamental importance to cell biology. Previous studies towards spatiotemporal control of tissue organisation have focused on modulation of the adhesive properties of coculture substrates, which included micropatterning of cells using appropriate surface chemistry.

Elliot E. Hui and Sangeeta N. Bhatia introduced in a recent work a different approach by designing a dynamic substrate, in which cell distances can be altered with micrometre-scale precision.¹ They developed a micromachined silicon substrate with moving parts to investigate the dynamic regulation of cell–cell interactions *via* direct control of cell positioning (Fig. 1). Thereby, they achieved mechanical control of both tissue composition and spatial organisation. The

microchip consists of two comb-like plates and an integrated snap-lock mechanism. The plates can be fully separated, slightly separated with a fixed gap, or locked together with the comb fingers in contact. Cells of different populations are grown on each plate, and the cell–cell contact and the extent of soluble signalling is regulated by positioning plates together or apart. As a case, the intercellular communication between hepatocytes and supportive stromal cell is investigated. It is known, that the coculture of primary hepatocytes with stromal cells (*e.g.* fibroblasts) promotes retention of hepatocyte viability and liver-specific functions. Although the requirement of coculture is an important finding for both therapeutic and diagnostic applications of engineered liver cells, the relative role of contact-mediated *versus* soluble factors for the cell–cell communication is poorly understood so far. The novel microchip facilitates such studies. Hepatocytes and fibroblast cells are cultured for two weeks on opposing comb plates, and the viability and morphology of the hepatocyte cells are observed microscopically. Additionally, albumin production is

measured as a quantitative marker of liver-specific function. Cultures in the contact, gap and separated mode are compared. Furthermore, dynamic experiments, in which the cell–cell distance is changed after 18 hours are performed. The results indicate that hepatocytes remain viable and functional if they are in direct contact to fibroblasts. A transient contact of cells proved sufficient, if it is followed by sustained soluble signal provided by culturing the cells in the gap mode of the device. Hereby, the distance of the hepatocytes and fibroblasts is critical, and has to be shorter than the effective range of soluble factors of $\sim 325 \mu\text{m}$.

The device can be reused multiple times, and is compatible with other cell types. The authors anticipate that this methodology of dynamical regulation of cell distances will find broad utility *e.g.* for the investigation of embryogenesis, homeostasis and pathogenic processes. Future directions of device engineering could include embedded microfluidic channels and integrated sensors for local delivery of soluble factors and *in situ* monitoring of cell viability and function.

Pesticide determination in ceramics microsystems

Among the several materials used for microsystem fabrication glass and polymers are most widely used as they are transparent and allow direct microscopic observation of the analytes. However, for certain applications, ceramics are becoming a good alternative due to some interesting advantages. Low-temperature cofired ceramics (LTCC) permits a fast and simple construction of three-dimensional structures, and different elements can be integrated and cofired at the same time. Due to its perfect compatibility with screen-printing techniques, it facilitates the integration of electronic components. Furthermore, perfect sealing can be achieved during the fabrication, thus rendering the use of glues or gaskets unnecessary. Julián Alonso and co-workers from the Universitat Autònoma de Barcelona (Spain)

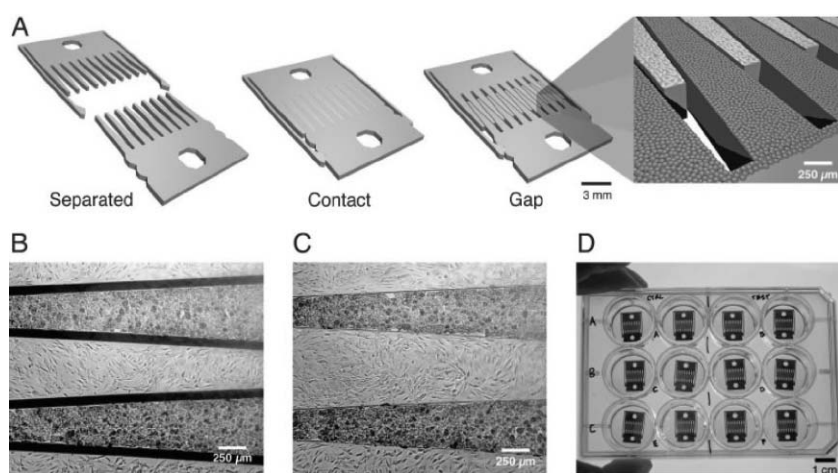


Fig. 1 Microchip for micrometre-resolution cell positioning. (A) Layout of the microchip that consists of two silicon parts that can be fully separated, locked together with the tapered comb fingers in contact, or slightly separated with a gap of a specific size using a snap-lock mechanism. On top of the silicon parts, cells can be cultured (right inset). (B) and (C) Different cell types (hepatocytes and fibroblasts) are cultured on the comb fingers. (D) The chips are positioned in a standard 12-well plate. (Reprinted from Hui and Bhatia¹ with permission. Copyright 2007 National Academy of Sciences, USA.)

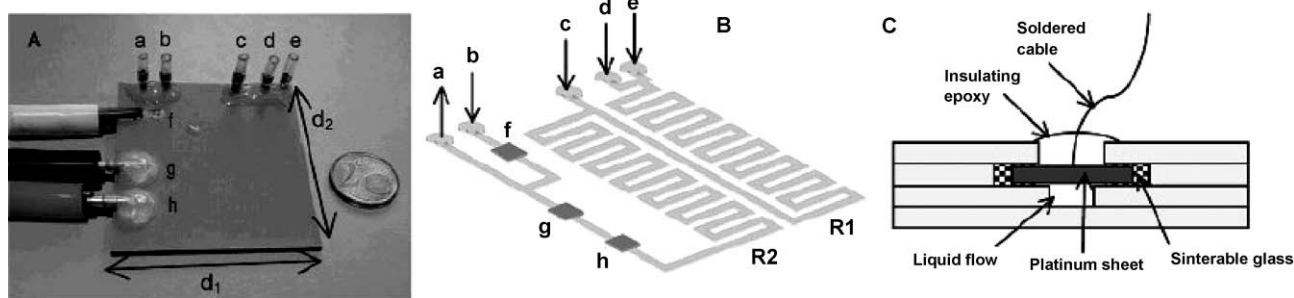


Fig. 2 Pesticide detection in a low-temperature cofired ceramics microsystem. (A) Picture of the device. (B) The inner three-dimensional structure with sample input and output channels (a–e), electrodes for amperometric detection (f–h) and two reactors (R1, R2). (C) Side view of the device. (Reprinted from Llopis *et al.*³ Copyright 2007 American Chemical Society.)

presented in a recent work the construction and use of a LTCC microsystem to determine pesticides.² The device integrates a pre-treatment stage with two mixers and reactors for incubation of the reaction mixture, and an amperometric detection system that is monolithically integrated in the microfluidic platform (Fig. 2). The detection system consists of two platinum sheets acting as counter and working electrodes, and a silver-based screen-printed conductor acting as reference electrode.

The ceramics microsystem is used to determine the pesticide carbofuran in water streams by conduction of an enzymatic inhibition reaction. The enzyme acetylcholinesterase that catalyzes the hydrolysis of acetylthiocholine is strongly inhibited by carbofuran. The product of the hydrolysis, thiocholine, is an electroactive substance and can be followed amperometrically. First, the sensitivity and reproducibility of the system toward thiocholine is characterized without enzymatic reaction. Second, a good correlation between the percentage of inhibition (%I) and carbofuran concentration is proven ($0.128\%I \text{ mM}^{-1}$) with a limit of detection of 36 nM carbofuran.

Glass blowing on the microscale

Glass blowing is an art that has been employed for more than 2000 years. Nowadays, it is widely used to fabricate scientific glassware, optical components, consumer glass containers, and visual arts. Such items have typically a size of several centimetres. A process for shaping glass shells with diameters less than 1 mm, as recently developed by

E. J. Eklund and A. M. Shkel, could enable novel capabilities such as mass-production of microscopic spherical gas chambers or microlenses (Fig. 3).³ The fabrication procedure relies on several steps: First, cavities are etched into a silicon wafer. Second, a thin glass wafer is bonded to the etched silicon wafer. Next, the bonded wafers are heated in a furnace for ~ 3 min. Upon heating of the wafers above the softening point of the glass (~ 850 °C), the glass is blown into spherical shells, due to the expansion of the trapped gas inside the cavities of the silicon wafer. Finally, the bonded wafers are removed quickly from the furnace so that the glass solidifies before the pressure inside the shells is reduced due to the lower temperature.

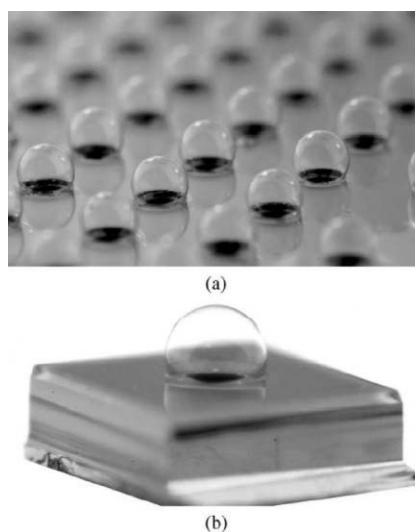


Fig. 3 Glass blowing on a wafer level. The spherical glass shells blown on a silicon wafer have diameters of less than a millimetre. (Reprinted with permission from Eklund and Shkel.³ Copyright 2007 IEEE.)

The glass shells can be produced in an array format on a single wafer. The height and radius of the glass shells varies depending on the experimental conditions such as the size of the etched cavity. Heating of a silicon wafer, *e.g.* with etched cavities of 250 μm (radius) \times 750 μm (depth) bonded to a 100 μm Pyrex glass wafer, results in a sphere with a height of about 818 μm and a radius of 431 μm . The thickness of the shell wall is not homogeneous; it is thinner at the top (5.3 μm) than at the side of the shell (11 μm). Several shells are gauged in this way, and the values are compared to theoretical values that are calculated using an analytical model of the process that has been developed by the authors. Several modifications of the process are suggested, *e.g.* to use a mould in order to shape non-spherical structures. Furthermore, the glass shells could be filled, *e.g.* by etching the back of the silicon wafer after the fabrication process.

Microchip electrophoretic immunoassay for rapid saliva-based diagnostic

Over the last decade, many microfluidic devices have been developed to analyze blood samples. Such devices are extremely versatile for point-of-care (POC) diagnostics due to a significant reduction of sample and reagent consumption, and due to the decrease of assay times.

In some recent studies, microsystem-based diagnostics are also employed for samples of saliva. It has been recognized that oral fluid protein content could mirror the development and progression of oral, as well as systematic diseases. One example is the periodontal disease

(periodontitis) that is initiated by tooth-associated pathogenic bacterial plaque biofilms and leads to chronic tissue injury. The matrix metalloproteinase-8 (MMP-8) has been identified as a major tissue destructing enzyme in periodontitis, and thus, a promising target molecule for diagnosis of the disease. In a current work, Anup K. Singh and co-workers have developed a microfluidic immunoassay to determine quantitatively the enzyme MMP-8 in saliva.⁴ The microchip for MMP-8 determination integrates sample pre-treatment (loading, enrichment, mixing, incubation) with subsequent quantitative immunoassay. Channel sections with distinct functionalities are created on the microchips by photopatterning polyacrylamide gels with specific physical properties: A gel with larger pore sizes for sample loading and mixing, a size-exclusion membrane for sample enrichment, and a small-pore size gel for electrophoretic

separation. During operation of the device, a detection mixture with fluorescently labelled monoclonal antibody (α MMP-8*) is loaded, followed by the introduction of saliva sample. Saliva and α MMP-8* are coenriched at the size-exclusion membrane. After a short incubation time of a few minutes, the enriched species are eluted into the separation channel by altering the electrical potentials. The fluorescent MMP-8 complex is detected at the end of the separation channel using laser-induced fluorescence. An estimate of the lower limit of quantitation of the method yields a value of 130 ng ml⁻¹. Comparison of the method with conventional ELISA (enzyme linked immunosorbent assay) shows that the microchip approach can be used to quantify MMP-8 concentration in saliva. Furthermore, the microdevice is used for quantitation of MMP-8 in saliva from healthy and periodontally diseased individuals. The results confirm

the applicability of the device for rapid POC diagnostics.

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